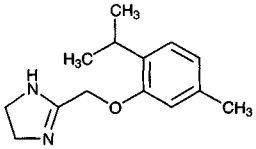
Mobile phase: Gradient. A was dichloromethane:MeOH 90:10. B was hexane. A:B from 10:90 to 90:10 over 18 min, return to initial conditions over 2 min.
Flow rate: 1
Detector: UV 280

REFERENCE

DeAngelis,R.L.; Kearney,M.F.; Barnes,E.R.; Shockcor,J.P.; Findlay,J.W.A. Balance/excretion of ^{3}H- and ^{14}C-tyloxapol in the male rabbit after intratracheal administration, *Xenobiotica*, **1995**, *25*, 521–530.

Tymazoline



Molecular formula: $C_{14}H_{20}N_2O$
Molecular weight: 232.33
CAS Registry No.: 24243-97-8
Merck Index: 9965

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 12 μm 1-myristoyl-2-[(13-carboxyl)-tridecoyl]-sn-3-glycerophosphocholine chemically bonded to silica (Regis)
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 20:80
Flow rate: 1
Detector: UV 254

CHROMATOGRAM

Retention time: k' 16.00

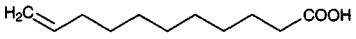
OTHER SUBSTANCES

Also analyzed: acebutolol, alprenolol, antazoline, atenolol, betaxolol, bisoprolol, bopindolol, bupranolol, carteolol, celiprolol, chloropyramine, chlorpheniramine, cicloprolol, cimetidine, cinnarizine, cirazoline, clonidine, dilevalol, dimethindene, diphenhydramine, doxazosin, esmolol, famotidine, isothipendyl, ketotifen, metiamide, metoprolol, moxonidine, nadolol, naphazoline, nifenalol, nizatidine, oxprenolol, pheniramine, phentolamine, pindolol, pizotyline (pizotifen), practolol, prazosin, promethazine, propranolol, pyrilamine (mepyramine), ranitidine, roxatidine, sotalol, tiamenidine, timolol, tramazoline, tripelennamine, triprolidine, UK-14,304

REFERENCE

Kaliszan,R.; Nasal,A.; Turowski,M. Binding site for basic drugs on α_1-acid glycoprotein as revealed by chemometric analysis of biochromatographic data, *Biomed.Chromatogr.*, **1995**, *9*, 211–215.

Undecylenic acid



Molecular formula: $C_{11}H_{20}O_2$
Molecular weight: 184.28
CAS Registry No.: 112-38-9, 557-08-4 (Zn salt)
Merck Index: 9983

SAMPLE

Matrix: blood
Sample preparation: Perform all operations with the exclusion of light. Evaporate 240 μL derivatization solution into a vial, add 400 μL 50 mM pH 7.0 phosphate buffer, add 100 μL

plasma, vortex for 5 s, heat at 70° for 40 min, add 500 μL MeCN, centrifuge at 3000 g for 5 min, inject a 20 μL aliquot. (Derivatization solution was 1.65 g Arkopal N-130 (a non-ionic surfactant, nonylphenol/13 unit chain polyoxyethylene) + 650 mg tetrahexylammonium bromide + 60 mg 4-bromomethyl-7-methoxycoumarin in 20 mL acetone.)

HPLC VARIABLES
Guard column: 10 × 3 5-20 μm LiChroprep RP-8
Column: 100 × 3 5 μm Chromspher C18
Mobile phase: Gradient. MeOH:water 80:20 for 3 min, then to 100:0 over 6 min, then held at 100:0 for 4 min.
Injection volume: 20
Detector: F ex 330 em 395

CHROMATOGRAM
Retention time: 7
Internal standard: undecylenic acid
Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Simultaneous: valproic acid
Noninterfering: phenobarbital, phenytoin, carbamazepine

KEY WORDS
plasma; metabolites; undecylenic acid is IS; derivatization

REFERENCE
van der Horst,F.A.; Eikelboom,G.G.; Holthuis,J.J. High-performance liquid chromatographic determination of valproic acid in plasma using a micelle-mediated pre-column derivatization, *J.Chromatogr.*, **1988**, *456*, 191–199.

SAMPLE
Matrix: blood
Sample preparation: Prepare ultrafiltrate from serum with an Amicon Centifree unit by centrifuging at 700 g for 10 min. 25 μL Ultrafiltrate + 475 μL, centrifuge. Remove 50 μL supernatant, add 100 μL 18-crown-6 solution, add 50 μL 1 mg/mL 4-bromomethyl-7-methoxycoumarin in MeCN, let stand in the dark at 65° for 30 min, inject a 5 μL aliquot. (Prepare 18-crown-6 solution by dissolving 100 mg potassium carbonate in 50 μL water, add 5 mL 20 mM 18-crown-6 in MeCN, sonicate for 30 min, add 5 mL MeCN.)

HPLC VARIABLES
Column: 100 × 2.1 5 μm HP Hypersil-ODS
Mobile phase: MeOH:water 80:20
Column temperature: 40
Flow rate: 0.3
Injection volume: 5
Detector: F ex 322 em 695

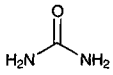
CHROMATOGRAM
Retention time: 4.5
Internal standard: undecylenic acid

OTHER SUBSTANCES
Simultaneous: valproic acid
Noninterfering: phenobarbital, phenytoin, carbamazepine

KEY WORDS
serum; undecylenic acid is IS; derivatization

REFERENCE
Liu,H.; Forman,L.J.; Montoya,J.; Eggers,C.; Barham,C.; Delgado,M. Determination of valproic acid by high-performance liquid chromatography with photodiode-array and fluorescence detection, *J.Chromatogr.*, **1992**, *576*, 163–169.

Urea



Molecular formula: CH_4N_2O
Molecular weight: 60.06
CAS Registry No.: 57-13-6
Merck Index: 10005

SAMPLE
Matrix: blood
Sample preparation: Dilute serum 200-fold with water. Add 50 μL 40 μg/mL uracil solution to 1 mL diluted serum, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 6.0 5 μm Sumipax ODS C-212 (Sumika Chemical Analysis Service, Japan)
Mobile phase: MeOH:water 15:85
Flow rate: 1
Injection volume: 10
Detector: MS, Finnigan MAT Model TSQ 700, APCI source 250°, vaporizer 400°, discharge current 5 μA, m/z 61, 62 and 113

CHROMATOGRAM
Retention time: 3.0 (^{12}C-urea, ^{13}C-urea)
Internal standard: uracil (3.7)
Limit of quantitation: 200 μg/mL (^{12}C-urea), 2 μg/mL (^{13}C-urea)

KEY WORDS
serum

REFERENCE
Tanigawa,T.; Mizo-oku,Y.; Moriguchi,K.; Suzuki,T.; Osumi,T.; Odomi,M. Simple and rapid quantitative assay of 13C-labelled urea in human serum using liquid chromatography-atmospheric pressure chemical ionization mass spectrometry, *J.Chromatogr.B*, **1996**, *683*, 135–142.

SAMPLE
Matrix: blood
Sample preparation: Inject a 500 μL aliquot of serum ultrafiltrate onto a 50 × 4.6 column packed with immobilized urease, let stand at room temperature for 10-15 min, inject another 100 μL ultrafiltrate to force ultrafiltrate into the 10 μL sample loop, inject this aliquot. (Prepare the urease column by suspending 1 g Eupergit C (epoxyacrylic resin granules, Röhm Pharma, Weiterstadt, Germany) in 2.5 mL 4 mg/mL urease (EC 3.5.1.5, Type IV, Jack Beans, Sigma) in water, filter after 1 h (Analyst 1984, 109, 147), suspend in water, slurry pack in a 50 × 4.6 column. The enzyme converts serum urea to ammonium ions which are then detected by the HPLC system. Wash column with pH 7 phosphate buffer after use, store in the refrigerator.)

HPLC VARIABLES
Column: 250 × 4.6 Wescan cation-exchange (Bio-Rad)
Mobile phase: Dilute phosphoric acid, pH 2.28
Flow rate: 1
Injection volume: 10
Detector: Conductivity

CHROMATOGRAM
Retention time: 4

KEY WORDS
derivatization; horse; serum; ultrafiltrate

REFERENCE
Shintani,H.; Ube,S. Simultaneous determination of serum cations, anions and uremic toxins by ion chromatography using an immobilized enzyme, *J.Chromatogr.*, **1985**, *344*, 145–156.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg Bond Elut SCX strong cation exchange (SO3H type) SPE cartridge with 2 mL MeOH and 2 mL 100 mM HCl. Filter (Amicon Centricon, cut-off 10000 daltons) while centrifuging at 4000 rpm, acidify ultrafiltrate to pH 3 with HCl, add 1 mL to the SPE cartridge, wash with 2 mL water, elute with 2 mL 1 M HCl at 0.3 mL/min, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 MCI CK 08S strong cation exchange SDB polymer base (SO3H type) (Mitsubishi Kasei)
Mobile phase: 1.5 mM HCl
Flow rate: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 24

KEY WORDS
whole blood; SPE; ultrafiltrate

REFERENCE
Shintani,H. Selection of columns for analysis of blood urea, *J.Liq.Chromatogr.*, **1994**, *17*, 1737–1742.

SAMPLE
Matrix: blood
Sample preparation: Condition a 500 mg 0.6 mL BondElut SCX SPE cartridge with 2 mL MeOH and 2 mL 100 mM HCl. Filter (Amicon Centricon, cut-off 10000 daltons) while centrifuging at 4000 rpm. Acidify the ultrafiltrate to pH 3 with HCl, add 1 mL to the SPE cartridge, wash with 2 mL water, elute with 2 mL 1 M HCl at 0.3 mL/min, inject an aliquot of the eluate.

HPLC VARIABLES
Column: 150 × 4.6 11-14 μm Mitsubishi Kasei MCI CK 08S strong cation exchange (SO3H type on 8% crosslinked DVB, 1.9 meq/mL)
Mobile phase: 1.5 mM HCl
Flow rate: 2
Detector: UV 210

CHROMATOGRAM
Retention time: 24

KEY WORDS
whole blood; SPE; ultrafiltrate

REFERENCE
Shintani,H. Solid phase extraction (SPE) of blood urea compared with liquid-liquid extraction regarding artifact formation, *J.Liq.Chromatogr.*, **1995**, *18*, 2167–2174.

SAMPLE
Matrix: blood
Sample preparation: Condition a 2.8 mL 500 mg Bond Elut SCX H type cation-exchange SPE cartridge with 3 mL MeOH and 3 mL water at 3 mL/min. Add blood, wash with 1 mL water at 3 mL/min, elute with 4 mL 5% phosphoric acid at 1 mL/min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 11-14 μm MCI GEL CK 08S polymer-based strong cation exchange (Na type) (Mitsubishi)
Mobile phase: 1 mM HCl
Column temperature: 35
Flow rate: 1

Injection volume: 20
Detector: UV 200

CHROMATOGRAM
Retention time: 6

KEY WORDS
SPE

REFERENCE
Shintani,H. Comparison of solid-phase extraction and dialysis on pretreatment efficiency of blood urea analysis, *J.Chromatogr.Sci.*, **1996**, *34*, 92–94.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute 100 (serum) or 1000 (urine) fold with water, filter (0.2 μm), adjust pH to 10-11 with KOH, add a 2 mL aliquot to the SPE column, discard the first 1.5 mL eluate, inject a 10 μL aliquot of the next eluate fraction. (Prepare the SPE column by adding 50-100 mesh Dowex 1-X2 strongly basic anion exchange resin to a Pasteur pipette (40 mm bed depth), add a few mL water to the column.)

HPLC VARIABLES
Guard column: 60 × 2 C18
Column: 150 × 3 5 μm Spherisorb ODS-2
Mobile phase: 50 mM pH 6.9 potassium phosphate buffer containing 5 mM sodium octylsulfonate
Flow rate: 0.5
Injection volume: 10
Detector: F ex 340 em 455 following post-column reaction. The column effluent passed through a 60 × 2 immobilized urease solid-phase reactor and then mixed with the reagent pumped at 0.5 mL/min. The mixture flowed through a coil of 0.2 mm i.d. PTFE tubing (volume 600 μL) to the detector. (Prepare reagent by dissolving 24.7 g boric acid in 1 L water, adjust pH to 10.2 with KOH, add 10 mL 80 mg/mL o-phthalaldehyde in EtOH, add 1 mL mercaptoethanol, store under nitrogen at 4°, stable for at least 1 week. Prepare the solid-phase reactor by treating 10 μm LiChrospher SI 500 with 3-aminopropyltriethoxysilane, couple urease (urea amidohydrolase EC 3.5.1.5, U-2000 (Sigma)) to the silica using 25% glutaraldehyde solution. Slurry the urease-silica in water and pack the reactor with 10 mM pH 6.9 potassium phosphate buffer at 110 bar for 15 min (J. Chromatogr. 1985, 325, 255). To maintain reactor performance flush system for 30 min with 50 mM pH 6.9 potassium phosphate buffer containing 5 mM EDTA every 2 weeks. Store reactor at 4° when not in use.)

CHROMATOGRAM
Retention time: 3.5
Limit of detection: 30 ppb

OTHER SUBSTANCES
Simultaneous: ammonia
Noninterfering: amino acids

KEY WORDS
serum; SPE; post-column reaction

REFERENCE
Jansen,H.; van der Velde,E.G.; Brinkman,U.A.T.; Frei,R.W. Liquid chromatographic determination of urea and ammonia in body fluids using a post-column enzymatic reactor, *J.Chromatogr.*, **1986**, *378*, 215–221.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 100 mg carbamide peroxide formulation, make up to 50 mL with water, mix, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 6.5 CHO-620 Carbohydrate (Interaction Chemicals Inc., Mountain View, CA)
Mobile phase: 50 μg/mL Calcium disodium EDTA in water
Column temperature: 85
Flow rate: 0.6
Injection volume: 20
Detector: UV 200, UV 210

CHROMATOGRAM
Retention time: 15
Limit of detection: 1 μg/g
Limit of quantitation: 15 μg/g

OTHER SUBSTANCES
Simultaneous: excipients

REFERENCE
Walker,T.A. A liquid chromatographic assay for urea in over-the-counter carbamide peroxide products, *J.Pharm.Biomed.Anal.*, **1994**, *12*, 1277–1282.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Polytron for >10 mg; Kontes micro-ultrasonic cell disrupter for <10 mg) tissue with 40 volumes 25 μg/mL β-aminoisobutyric acid in EtOH:water:glacial acetic acid 75:20:5, centrifuge at 4° at 25000 g for 20 min. Remove a 50 μL aliquot of the supernatant and evaporate it to dryness under reduced pressure, suspend the residue in 100 μL 100 mM sodium bicarbonate by sonicating or vortexing, add 200 μL 1.25 mg/mL dansyl chloride in acetone, vortex, heat at 90° for 30 min, centrifuge at 5000 g for 20 min, inject a 4 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 75 × 4.6 3 μm Ultrasphere ODS
Mobile phase: MeCN:water:phosphoric acid 13:87:0.15
Flow rate: 1
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: k' 2.45
Internal standard: β-aminoisobutyric acid (k' 9.25)
Limit of quantitation: 10 pmole

OTHER SUBSTANCES
Extracted: amino acids, taurine
Interfering: arginine, hydroxyproline, homocarnosine

KEY WORDS
rat; brain; derivatization

REFERENCE
Saller,C.F.; Czupryna,M.J. γ-Aminobutyric acid, glutamate, glycine and taurine analysis using reversed-phase high-performance liquid chromatography and ultraviolet detection of dansyl chloride derivatives, *J.Chromatogr.*, **1989**, *487*, 167–172.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 500 mg washed and mixed Duolite ion-exchange resin (BDH), vortex for 10 s, centrifuge at 3000 g for 10 min, filter (0.2 μm) the supernatant, inject an aliquot.

HPLC VARIABLES
Guard column: Direct-Connect polymeric guard column (Alltech)
Column: 250 × 4.6 5 μm Kromasil NH2 (Alltech)
Mobile phase: MeCN:water 70:30
Flow rate: 1
Injection volume: 10
Detector: RI

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Extracted: lactulose, mannitol, L-rhamnose

REFERENCE
Miki,K.; Butler,R.; Moore,D.; Davidson,G. Rapid and simultaneous quantification of rhamnose, mannitol, and lactulose in urine by HPLC for estimating intestinal permeability in pediatric practice, *Clin.Chem.*, **1996**, *42*, 71–75.

Urokinase

Molecular weight: 30000-50000
CAS Registry No.: 9039-53-6
Merck Index: 10024

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 7.5 TSK G3000SW (Alltech)
Mobile phase: 100 mM KH_2PO_4 containing 100 mM NaCl and 5 mM sodium azide, adjusted to pH 6.0 with 10 M NaOH
Flow rate: 0.5
Injection volume: 200
Detector: UV 280

CHROMATOGRAM
Retention time: 19

REFERENCE
Cox,R.A.; McFarland,K.N.; Sackett,P.H.; Short,M.T. Correlation of urokinase activity from biopotency and high-performance liquid chromatographic assays, *J.Chromatogr.*, **1986**, *370*, 495–500.

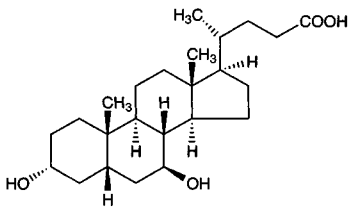
SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Bakerbond C4
Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 0:100 to 60:40 over 90 min
Flow rate: 1
Injection volume: 200
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: alteplase, streptokinase, anistreplase

REFERENCE
Werner,R.G.; Bassarab,S.; Hoffmann,H.; Schlüter,M. Quality aspects of fibrinolytic agents based on biochemical characterization, *Arzneimittelforschung*, **1991**, *41*, 1196–1200.

Ursodiol



Molecular formula: $C_{24}H_{40}O_4$
Molecular weight: 392.58
CAS Registry No.: 128-13-2
Merck Index: 10026

SAMPLE
Matrix: bile
Sample preparation: Extract bile with 20 volumes EtOH, boil on a hot water bath, cool, let stand overnight, filter (Toyo Roshi 5A paper), filter (0.45 μm), add 200 μg/mL testosterone acetate in EtOH (final IS concentration 100 μg/mL), inject a 5-10 μL aliquot.

HPLC VARIABLES
Guard column: Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:MeOH:30 mM phosphate buffer 10:60:30, pH 3.40
Flow rate: 0.5
Injection volume: 5-10
Detector: UV 200

CHROMATOGRAM
Retention time: 12 (taurine conjugate), 13 (glycine conjugate)
Internal standard: testosterone acetate (39)
Limit of detection: 50 ng

OTHER SUBSTANCES
Extracted: chenodiol, conjugates, bile acids, deoxycholic acid

REFERENCE
Nakayama,F.; Nakagaki,M. Quantitative determination of bile acids in bile with reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 287–293.

SAMPLE
Matrix: bile, blood
Sample preparation: Serum. 100-200 μL Serum + 1 mL MeOH, mix, sonicate for 15 min. Remove a 600 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute with 1 mL 50 mM pH 7.0 phosphate buffer, add to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 μL aliquot and add it to 50 μL 100 μM lauric acid in MeOH, add 50 μL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 μL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 μL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 μL aliquot. Bile. Mix 10 μL bile with 10 mL 50 mM pH 7.0 phosphate buffer, add a 1 mL aliquot to a Sep-Pak C18 SPE cartridge, wash with 2 mL MeOH:water 20:80, elute with 4 mL MeOH:water 80:20. Evaporate the eluate to dryness under reduced pressure at 40°, reconstitute with 1 mL MeOH. Remove a 500 μL aliquot and add it to 50 μL 100 μM lauric acid in MeOH, add 50 μL 0.1 mg/mL KOH on MeOH, evaporate to dryness under a stream of nitrogen, add 100 μL 1 mg/mL dicyclohexyl-18-crown-6 in MeCN, add 100 μL 25 mM 1-bromoacetylpyrene in MeCN, mix, heat at 40° for 30 min, cool, inject an 8 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 10 μm Model RCM-100 Radial-Pak A (Waters)

Mobile phase: Gradient. MeCN:MeOH:water 100:50:40 for 30 min then 100:50:20 (step gradient).
Flow rate: 2
Injection volume: 8
Detector: F ex 370 em 440

CHROMATOGRAM

Retention time: 26
Internal standard: lauric acid (56)
Limit of detection: 10 pmole
Limit of quantitation: 50 pmole

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid

KEY WORDS

derivatization; serum; SPE

REFERENCE

Kamada,S.; Maeda,M.; Tsuji,A. Fluorescence high-performance liquid chromatographic determination of free and conjugated bile acids in serum and bile using 1-bromoacetylpyrene as a pre-labeling reagent, *J.Chromatogr.*, **1983**, *272*, 29–41.

SAMPLE

Matrix: bile, blood, feces, gastric contents, tissue
Sample preparation: Condition a Sep-Pak C18 cartridge with 2 mL 720 mM MeOH in water and 6 mL 100 mM pH 7.0 potassium phosphate buffer. Serum. 200 μL Serum + 1 mL MeCN, mix, sonicate for 10 min, centrifuge at 17000 g for 15 min. Remove a 600 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen at 75°, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Liver. Homogenize (glass homogenizer) liver in 1 mL 720 mM EtOH in water, add 2 mL 720 mM EtOH in water, heat at 75° for 15 min, centrifuge at 17000 g for 10 min, remove the supernatant, extract the residue twice more. Combine the supernatants and evaporate them to dryness at 75°, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Bile. Dilute 20 μL bile with 10 mL 100 mM pH 7.0 potassium phosphate buffer. Add 1 mL to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Gastric juice. Dilute 1 mL gastric juice with 9 mL 100 mM pH 7.0 potassium phosphate buffer, sonicate for 10 min. Add 1 mL to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, reconstitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot. Feces. Dilute 1 g feces with 9 mL MeOH, mix thoroughly, sonicate for 10 min, centrifuge at 17000 g for 10 min. Remove a 1 mL aliquot of the supernatant and evaporate it to dryness, reconstitute with 5 mL 100 mM pH 7.0 potassium phosphate buffer. Add to the SPE cartridge at 0.5 mL/min, wash with 2 mL 40 mM MeOH in water, elute with 4 mL 720 mM MeOH in water, filter (0.45 μm), evaporate the filtrate to dryness, recon-

stitute with 50 μL 250 μM lauric acid in MeOH, add 50 μL 1.8 mM KOH in MeOH, evaporate to dryness under a stream of nitrogen at 75°, reconstitute with 100 μL 10 mM 4-bromomethyl-7-methoxycoumarin in MeCN containing 5 mM dicyclohexyl-18-crown-6, let stand at room temperature for 35 min, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere I.P. C18
Mobile phase: Gradient. A was MeCN:MeOH:water 100:50:75. B was MeCN:MeOH 100:50. A:B 100:0 for 7 min, to 70:30 over 0.5 min, maintain at 70:30 for 5 min, to 50:50 over 0.5 min, maintain at 50:50 over 7 min, to 25:75 over 1 min, maintain at 25:75 for 7 min.
Column temperature: 35
Flow rate: 1.7
Injection volume: 100
Detector: F

CHROMATOGRAM

Retention time: 15
Internal standard: lauric acid (24.5)
Limit of detection: 0.5 pmole

OTHER SUBSTANCES

Extracted: chenodiol (chenodeoxycholic acid), cholic acid, deoxycholic acid, glycinechenodeoxycholic acid, glycinecholic acid, glycinedeoxycholic acid, glycinelithocholic acid, glycineursodeoxycholic acid, lithocholic acid

KEY WORDS

derivatization; SPE; liver; serum

REFERENCE

Güldütuna,S.; You,T.; Kurts,W.; Leuschner,U. High performance liquid chromatographic determination of free and conjugated bile acids in serum, liver biopsies, bile, gastric juice and feces by fluorescence labeling, *Clin.Chim.Acta*, **1993**, *214*, 195–207.

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Urine. Condition a Bond Elut C18 SPE cartridge with MeOH and water. Dilute 100-200 μL urine 1:4 with 100 mM NaOH, add to the SPE cartridge, wash with water, elute with MeOH, evaporate the eluate, reconstitute the residue in mobile phase, inject an aliquot. Serum. Condition a Bond Elut C18 SPE cartridge with MeOH and water. Dilute 100-500 μL serum with 3.5 mL 100 mM NaOH, heat at 64° for 30 min, add to the SPE cartridge, wash with water, elute with MeOH, evaporate the eluate, reconstitute the residue in mobile phase, inject an aliquot. Bile. Dilute 1:500 to 1:1000 with mobile phase, filter (0.22 μm), inject an aliquot.

HPLC VARIABLES

Column: 70 × 4.6 3 μm Ultrasphere XL C18
Mobile phase: MeOH:15 mM ammonium acetate 80:20, apparent pH 6.0 ± 0.1
Flow rate: 0.3
Detector: MS, electrospray, Fisons VG TRIO 2000 quadrupole (6% of the mobile phase was diverted to the MS detector) or evaporative light scattering detector (Varex)

CHROMATOGRAM

Retention time: 7.75
Limit of detection: 15 pg

OTHER SUBSTANCES

Extracted: chenodiol, deoxycholic acid, bile acids, cholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycoursodeoxycholic acid, lithocholic acid, taurochenodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, tauroursodeoxycholic acid

KEY WORDS

serum; SPE; hamster; human; LC-MS

REFERENCE

Roda,A.; Gioacchini,A.M.; Cerrè,C.; Baraldini,M. High-performance liquid chromatographic-electrospray mass spectrometric analysis of bile acids in biological fluids, *J.Chromatogr.B*, **1995**, *665*, 281–294.

SAMPLE

Matrix: bile, formulations

Sample preparation: Bile. Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Condition a 500 mg Bond Elut SAX SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL MeOH. 50 μL Bile + 5 mL 50 mM pH 7.5 phosphate buffer, vortex, add to the C18 SPE cartridge, wash with 5 mL MeOH:40 mM pH 4.3 acetate buffer 40:60, wash with 10 mL water, elute with 2 mL MeOH. Add the eluate to the SAX SPE cartridge, elute with 3.5 mL MeOH, collect all the effluent from the cartridge (J. Pharm. Biomed. Anal. 1990, 8, 235). Evaporate to dryness under a stream of nitrogen, reconstitute with 2 mL MeOH, sonicate at 40° for 3 min, filter (0.2 μm). Add a 500 μL aliquot of the filtrate to 50 μL 0.01% KOH in MeOH, evaporate to dryness, reconstitute with 200 μL MeOH:water 10:90, sonicate at 40° for 3 min, add 300 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer, add 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate at 40° for 10 min, add 50 μL 43.6 μg/mL IS in MeOH:water 75:25, add 300 μL MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. Formulations. Powder capsule contents, weigh out amount containing about 25 mg compound, add 100 mL MeOH (water for bile acid salts), stir for 10 min, filter, dilute the filtrate 10-fold with water (or MeOH:water 10:90 for bile acid salts). Evaporate 50 μL 0.01% KOH in MeOH in to a tube, add a 200 μL aliquot of the diluted filtrate, add 300 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer, add 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, sonicate at 40° for 10 min, add 50 μL 43.6 μg/mL IS in MeOH:water 75:25, add 300 μL MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform: petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 5 μm Hypersil RP-18

Mobile phase: Gradient. For bile use MeCN:water 60:40 for 10 min, to 80:20 over 10 min, maintain at 80:20 for 25 min, return to initial conditions over 5 min. For formulations use isocratic MeCN:water 78:22.

Flow rate: 1

Injection volume: 50

Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 18 (gradient), 7 (isocratic)

Internal standard: 6-methoxynaphthacyl ester of valproic acid (23 (gradient), 10.5 (isocratic))

Limit of detection: 1-2 pmole

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycolithocholic acid, glycoursodeoxycholic acid, lithocholic acid

KEY WORDS

derivatization; capsules; SPE

REFERENCE

Cavrini,V.; Gatti,R.; Roda,A.; Cerrè,C.; Roveri,P. HPLC-fluorescence determination of bile acids in pharmaceuticals and bile after derivatization with 2-bromoacetyl-6-methoxynaphthalene, *J.Pharm.Biomed.Anal.*, **1993**, *11*, 761–770.

SAMPLE

Matrix: bile, gastric contents

Sample preparation: Condition a 200 mg Bond Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Condition a 500 mg Bond Elut SAX SPE cartridge with 5 mL MeOH, 5 mL water, and 5 mL MeOH. Mix 50 μL bile or 500 μL gastric juice with 5 mL 50 mM pH 7.5 phosphate

buffer, vortex, add to the C18 SPE cartridge, wash with 5 mL MeOH:40 mM pH 4.3 acetate buffer 40:60, wash with 10 mL water, elute with 2 mL MeOH. Add the eluate to the SAX SPE cartridge, elute with 3.5 mL MeOH, collect all the effluent from the cartridge. Evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL initial mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeOH:30 mM sodium acetate 65:35, adjusted to pH 4.3 with phosphoric acid. B was MeOH:70 mM sodium acetate 90:10, adjusted to pH 4.3 with phosphoric acid. A:B 85:15 for 10 min, to 10:90 over 25 min, maintain at 10:90 for 5 min.
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 18

OTHER SUBSTANCES
Extracted: cholic acid, chenodiol, deoxycholic acid, lithocholic acid

KEY WORDS
SPE

REFERENCE
Scalia,S. Group separation of free and conjugated bile acids by pre-packed anion-exchange cartridges, *J.Pharm.Biomed.Anal.*, **1990**, *8*, 235–241.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2 mL MeOH, 10 mL water, and 2 mL 100 mM pH 8.0 Tris-HCl buffer. 5-7 mL Serum + 19 volumes 100 mM pH 8.0 Tris-HCl buffer, sonicate for 10 min, add to the SPE cartridge, wash with 15 mL water, elute with 6-7 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 50°, dissolve residue in water, filter (Millipore GS 0.22 μm), wash filter, evaporate filtrates to dryness, reconstitute in 100 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:20 mM KH_2PO_4 65:35, adjust pH to 5.3
Flow rate: 1.4
Injection volume: 30
Detector: UV 210

CHROMATOGRAM
Retention time: 6 (taurine conjugate), 7 (glycine conjugate)

OTHER SUBSTANCES
Extracted: conjugates, chenodiol, bile acids, deoxycholic acid

KEY WORDS
serum; SPE

REFERENCE
Linnet,K. A high-pressure liquid chromatogaphic-enzymatic assay for glycine and taurine conjugates of cholic, chenodeoxycholic and deoxycholic acid in serum, *Scand.J.Clin.Lab.Invest.*, **1982**, *42*, 455–460.

SAMPLE
Matrix: blood
Sample preparation: Condition a BondElut SPE cartridge with 5 mL EtOH and 5 mL water. 100 μL Serum + 250 ng deoxycholic acid 12-propionate + 1 mL 500 mM pH 7.0 phosphate

buffer, mix, add to the SPE cartridge, wash with 2 mL water, wash with 1 mL 1.5% EtOH, elute with 2 mL 90% EtOH. Evaporate a 400 μL aliquot of the eluate, add 100 μL 2 mg/mL 1-anthroyl nitrile in MeCN, add 0.16% quinuclidine in MeCN, heat at 60° for 20 min, add 50 μL MeOH, evaporate under nitrogen. Dissolve the residue in 1 mL 90% EtOH, add to a 18 × 6 100 mg column of PHP-LH-20 Sephadex at 0.2 mL/min, wash with 1 mL 90% EtOH, elute with 5 mL 100 mM acetic acid in 90% EtOH (free bile acids), elute with 5 mL 200 mM formic acid in 90% EtOH (glycine-conjugated bile acids), elute with 5 mL 300 mM pH 6.3 acetic acid-potassium acetate in 90% EtOH (taurine-conjugated bile acids). Evaporate each fraction, dissolve the residue in 100-200 μL MeOH, inject a 5-10 μL aliquot. (Preparation of PHP-LH-20 Sephadex is as follows. Suspend 75.7 g Sephadex LH-20 in 200 mL dichloromethane using a glass stirring rod (not a magnetic stirrer) for 30 min, add 19 mL boron trifluoride ethyl etherate, after 15 min add 50 mL 35% epichlorohydrin in dichloromethane at 1-2 mL/min (Caution! Epichlorohydrin is a carcinogen!), stir for another 30 min, filter, wash with EtOH, dry chlorohydroxypropyl Sephadex LH-20 at 50° (J.Chromatogr. 1971, 59, 45). Stir 27.2 g chlorohydroxypropyl Sephadex LH-20 in 100.5 mL piperidine at room temperature for 30 min, add 5.74 g KOH in 302 mL MeOH, heat at 50-60° for 3 h with occasional shaking, filter, wash with EtOH:water 50:50, wash with 200 mM acetic acid in EtOH:water 70:30, wash with EtOH:water 90:10 until washings become neutral, store in EtOH:water 90:10 (Clin. Chim. Acta 1978 87 141). Prepare 1-anthroyl nitrile as follows. Dissolve 50 g benzanthrone in 500 mL concentrated sulfuric acid with gentle warming, pour this solution cautiously into 4 L hot water with vigorous stirring. Boil the suspension and slowly add 200 g chromium(VI) oxide (Caution! Chromium oxide is a carcinogen and highly corrosive!), after 6 h cool the mixture, filter, wash the precipitate with hot water. Dissolve the precipitate in dilute ammonia and precipitate with acid, crystallize from boiling concentrated nitric acid to give anthraquinone-1-carboxylic acid (Ber. 1924, 57, 1775). Warm, on a water bath, anthraquinone-1-carboxylic acid in dilute ammonia with twice the amount of zinc dust, when the reaction has ceased (30 min ?) filter the reaction the reaction mixture, add HCl to the filtrate to obtain anthracene-1-carboxylic acid as yellow needles, recrystallize from EtOH (mp 245°) (Ber 1897, 30, 1118). Stir 1 g anthracene-1-carboxylic acid in 15 mL anhydrous dichloromethane, add 2 mL oxalyl chloride, reflux for 1 h, evaporate to give 1-anthroyl chloride as an oily residue. Dissolve 1-anthroyl chloride in 15 mL dichloromethane, add 3 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, recrystallize from hexane/dichloromethane to give 1-anthroyl nitrile as orange-yellow needles (mp 164-5°) (Anal.Chim.Acta 1983, 147, 397).)

HPLC VARIABLES

Column: 150 × 4 5 μm Cosmosil 5C18
Mobile phase: MeOH:0.3% pH 6.0 potassium phosphate buffer 5:1
Flow rate: 1.8
Injection volume: 10
Detector: F ex 370 em 470

CHROMATOGRAM

Retention time: 10
Internal standard: deoxycholic acid 12-propionate (20)
Limit of detection: 50 nM

OTHER SUBSTANCES

Extracted: chenodiol, cholic acid deoxycholic acid, conjugates

KEY WORDS

serum; SPE; derivatization

REFERENCE

Goto,J.; Saito,M.; Chikai,T.; Goto,N.; Nambara,T. Studies on Steroids. CLXXXVII. Determination of serum bile acids by high-performance liquid chromatography with fluorescence labeling, *J.Chromatogr.*, **1983**, *276*, 289–300.

SAMPLE

Matrix: blood
Sample preparation: Add 1 mL serum to a Waters C18 SPE cartridge, wash with two 4 mL portions of water, wash with two 2 mL portions of MeOH:water 10:90, wash with two 2 mL portions of MeOH:water 20:80, wash with two 2 mL portions of MeOH:water 30:70, wash with two 2 mL portions of MeOH:water 50:50, elute with 3 mL MeOH. Evaporate the eluate to

dryness under a stream of nitrogen at 80°, reconstitute the residue in 50 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Lichrosorb RP 18
Mobile phase: MeOH:30 mM KH_2PO_4 76:24
Flow rate: 1.2
Injection volume: 20
Detector: UV 201

CHROMATOGRAM
Retention time: 6
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Extracted: chenodiol (chenodeoxycholic acid)

KEY WORDS
serum; SPE

REFERENCE
Baillet-Guffroy,A.; Baylocq,D.; Rabaron,A.; Pellerin,F. Nuclear magnetic resonance spectrometry and liquid chromatography of two bile acid epimers: ursodeoxycholic and chenodeoxycholic acid, *J.Pharm.Sci.*, **1984**, *73*, 847–849.

SAMPLE
Matrix: blood
Sample preparation: Deproteinize 20 μL serum with a pretreatment column (Autoserumout, Sekisui), inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Medipola Bile column (Sekisui)
Mobile phase: Gradient. A was MeCN:MeOH:30 mM ammonium acetate 20:20:60. B was MeCN:MeOH:30 mM ammonium acetate 30:30:40. A:B from 100:0 to 80:20 over 10 min, to 0:100 over 27 min, maintain at 0:100 for 30 min.
Flow rate: 1
Detector: F ex 340 em 460 following post-column reaction detection. The effluent from the column was mixed with reagent pumped at 1 mL/min, the mixture flowed through a 20 × 4 3α-HSD column (Sekisui) containing bound 3α-hydroxysteroid dehydrogenase to the detector. (The reagent was 1.36 g/L KH_2PO_4, 372 mg/L disodium EDTA, 140 mg/L βNAD, and 450 μL/L 2-mercaptoethanol in water adjusted to pH 7.8 with 5 M KOH.)

CHROMATOGRAM
Retention time: 17

OTHER SUBSTANCES
Extracted: chenodiol, deoxycholic acid, bile acids

KEY WORDS
post-column reaction; immobilized enzyme reactor; serum

REFERENCE
Adachi,Y.; Nanno,T.; Itoh,T.; Kurumi,Y.; Yamazaki,K.; Sawada,Y.; Yamamoto,T. Determination of individual serum bile acids in chronic liver diseases: fasting levels and results of oral chenodeoxycholic acid tolerance test, *Gastroenterol.Jpn.*, **1988**, *23*, 401–407.

SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Dilute 100-200 μL serum with 4 mL 400 mM sodium bicarbonate, add to the SPE cartridge,

wash with 20 mL water, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 45°, reconstitute the residue in 100 μL 2 mg/mL 4-bromomethyl-7-methoxycoumarin in MeCN, add 400 μg sodium carbonate, add 50 μL 20 mg/mL 18-crown-6 in MeCN, heat at 40° for 1 h, make up to 500 μL with MeCN, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 3.9 5 μm Nova-Pak ODS
Mobile phase: Gradient. A was MeCN:MeOH:water 15:13.8:71.2. B was MeCN. A:B from 100:0 to 37:63 over 47 min (Waters convex curve + 2), to 0:100 over 0.1 min (Waters curve +9), maintain at 0:100 for 7.9 min, re-equilibrate at initial conditions for 6 min.
Flow rate: 1 for 47 min then 1.5
Injection volume: 10
Detector: F ex 320 em 385

CHROMATOGRAM
Retention time: 36.43
Limit of detection: 50 nM

OTHER SUBSTANCES
Extracted: chenodiol, cholic acid, deoxycholic acid, glycochenodeoxycholic acid, glycocholic acid, glycodeoxycholic acid, glycolithocholic acid

KEY WORDS
derivatization; serum; SPE

REFERENCE
Wang,G.F.; Stacey,N.H.; Earl,J. Determination of individual bile acids in serum by high performance liquid chromatography, *Biomed.Chromatogr.*, **1990**, *4*, 136–140.

SAMPLE
Matrix: bulk
Sample preparation: Add 5 mL of a 39.2 mg/mL solution in dry MeCN to 120 mg 1-(2,5-dihydroxyphenyl)-2-bromoethane and 100 μL triethylamine, heat at 70° for 2 h, dilute with 20 mL water, extract 3 times with diethyl ether. Combine the extracts and wash them with saturated sodium bicarbonate and water, dry over anhydrous sodium sulfate, evaporate, reconstitute, inject a 5 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethane is as follows. Slowly add 2.5 g phenyltrimethylammonium tribromide to a solution of 2',5'-dihydroxyacetophenone in 20 mL dry THF, stir at room temperature overnight (check by TLC with cyclohexane:ethyl acetate 70:30). Remove the precipitate by filtration and dry under reduced pressure, chromatograph using cyclohexane:ethyl acetate 70:30 to give 1-(2,5-dihydroxyphenyl)-2-bromoethane.)

HPLC VARIABLES
Guard column: 4 × 4 5 μm 5 μm Hypersyl ODS RP-18
Column: 100 × 4.6 3 μm Adsorbosphere
Mobile phase: MeCN:MeOH:100 mM pH 6.5 sodium acetate buffer 20:60:20
Flow rate: 1
Injection volume: 5
Detector: E, ESA Coulochem Model 5100A, Model 5010 analytical cell, porous graphite electrodes +0.6 V

CHROMATOGRAM
Retention time: 4.07
Limit of detection: 0.88 nM

OTHER SUBSTANCES
Simultaneous: chenodiol

KEY WORDS
derivatization

REFERENCE

Bousquet,E.; Santagati,N.A.; Tirendi,S. Determination of chenodeoxycholic acid in pharmaceutical preparations of ursodeoxycholic acid by high performance liquid chromatography with coulometric electrochemical detection, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 757–770.

SAMPLE

Matrix: formulations

Sample preparation: Mix a 750 μL aliquot of the liquid formulation with 3 mL MeOH, shake vigorously for 15 s, centrifuge at 1000 rpm for 5 min, inject a 20 μL aliquot of the supernatant.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Altima C18 (Alltech)

Mobile phase: MeCN:buffer 55:45 (Buffer was 10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid.)

Column temperature: 40

Flow rate: 1

Injection volume: 20

Detector: UV 201

KEY WORDS

liquid formulations

REFERENCE

Mallett,M.S.; Hagan,R.L.; Peters,D.A. Stability of ursodiol 25 mg/mL in an extemporaneously prepared oral liquid, *Am.J.Health-Syst.Pharm.*, **1997**, *54*, 1401–1404.

SAMPLE

Matrix: formulations

Sample preparation: Dilute with MeOH to an ursodiol concentration of 1.5 mg/mL, filter (0.22 μm), inject a 15 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Spheri-5 ODS C18

Mobile phase: MeOH:10 mM potassium phosphate buffer 75:25, pH adjusted to 5.25 with dilute phosphoric acid

Flow rate: 1.2

Injection volume: 15

Detector: UV 201

CHROMATOGRAM

Retention time: 8

OTHER SUBSTANCES

Simultaneous: degradation products

KEY WORDS

syrup; suspensions; stability-indicating

REFERENCE

Johnson,C.E.; Nesbitt,J. Stability of ursodiol in an extemporaneously compounded oral liquid, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 1798–1800.

SAMPLE

Matrix: solutions

Sample preparation: Mix an aliquot of solution (or hydrolyzed bile) with a 50% molar excess of triethylamine in MeCN, warm briefly, add a 50% molar excess of 100 mM 2-bromoacetophenone in MeCN, heat at 80-90° for 45-60 min, evaporate to dryness, reconstitute with dioxane (Caution! Dioxane is a carcinogen!), filter (0.47 μm), inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10/25 ODS

Mobile phase: Gradient. n-Heptane:dioxane 90:10 for 3 min then n-heptane:dioxane:isopropanol 70:25:5 (step gradient). (Caution! Dioxane is a carcinogen!)
Flow rate: 1.2
Detector: UV 254

CHROMATOGRAM
Retention time: 19
Limit of quantitation: 5 pmole

OTHER SUBSTANCES
Simultaneous: chenodiol, cholic acid, deoxycholic acid, hyodeoxycholic acid, lithocholic acid

KEY WORDS
derivatization

REFERENCE
Stellaard,F.; Hachey,D.L.; Klein,P.D. Separation of bile acids as their phenacyl esters by high-pressure liquid chromatography, *Anal.Biochem.*, **1978**, *87*, 359–366.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in mobile phase to a concentration of 1 mg/mL.

HPLC VARIABLES
Column: 100 mm long 5 μm C18
Mobile phase: MeOH:10 mM KH_2PO_4 65:35, pH 7.0
Flow rate: 1
Injection volume: 0.5
Detector: UV 200

CHROMATOGRAM
Retention time: k' = 3.66

OTHER SUBSTANCES
Simultaneous: chenodiol

REFERENCE
Roda,A.; Minutello,A.; Angellotti,M.A.; Fini,A. Bile acid structure-activity relationship: evaluation of bile acid lipophilicity using 1-octanol/water partition coefficient and reverse phase HPLC, *J.Lipid Res.*, **1990**, *31*, 1433–1443.

SAMPLE
Matrix: solutions
Sample preparation: Sample + 400 μL 5 mM DBD-PZ + 70 mM diethylphosphorocyanidate in MeCN, react for 6 h, inject a 1 μL aliquot. (Synthesis of 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole (DBD-PZ) is as follows. Dissolve 0.5 g magnesium sulfate heptahydrate and 6 g NaOH in 60 mL water, throughout the reaction keep the flask at about 20° with cold water cooling, add 15 mL 30% hydrogen peroxide, add 75 mL MeOH, add 12.1 g powdered benzoyl peroxide in one go, stir for 10 min, pour into 150 mL 20% sulfuric acid, extract three times with 50 mL portions of chloroform, determine peroxybenzoic acid concentration by iodometric titration (Tetrahedron 1967, 23, 3327). Slowly add 110 mL 1 M peroxybenzoic acid in chloroform to 7 g 2,6-difluoroaniline dissolved in 100 mL chloroform, stir at room temperature, when reaction is complete (iodometric titration) wash with 2% sodium thiosulfate, wash with 5% sodium carbonate, wash with water, dry over anhydrous sodium sulfate, evaporate to dryness under reduced pressure, recrystallize 2,6-difluoronitrosobenzene form EtOH (mp 108.5-109.5). Stir 8.5 g 2,6-difluoronitrosobenzene in 85 mL DMSO at room temperature and add a solution of 3.91 g sodium azide in 85 mL DMSO dropwise, let stand for about 1 h, add to a large volume of water, extract with ether, dry the extracts over anhydrous sodium sulfate, evaporate to dryness under reduced pressure and distil to give 4-fluoro-2,1,3-benzoxadiazole as a colorless oil (bp 83°/12 mm Hg) (J.Chem.Soc.(C) 1970, 1433). Add 11 mL chlorosulfonic acid dropwise to 3 g 4-fluoro-2,1,3-benzoxadiazole in 10 mL chloroform at 0-10°

(use a calcium chloride drying tube), stir at room temperature for 1 h, reflux for 2 h, cool, slowly pour into ice water, remove the organic layer, extract the aqueous layer with chloroform, combine the organic layer, wash, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, take up the residue in 5 mL benzene (Caution! Benzene is a carcinogen!), chromatograph on a 150 × 30 column of silica gel (100-200 mesh Kanto Chemical) with n-hexane:benzene 50:50, evaporate the appropriate fractions to give 4-(chlorosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (CBD-F) as pale yellow needles (mp 64-66°) (Anal. Chem. 1984. 56, 2461). Stir 0.76 g CBD-F in 70 mL MeCN at 0-10° and add 1 g dimethylamine hydrochloride in 10 mL 100 mM pH 10 borax dropwise, adjust pH to 5 with 1 M HCl, concentrate to about 10 mL under reduced pressure, extract three times with 200 mL portions of diethyl ether, wash with water, dry over anhydrous magnesium sulfate, evaporate under reduced pressure, chromatograph on a 500 × 20 column of silica gel with chloroform, isolate the appropriate fraction and re-chromatograph on the same column with ethyl acetate:benzene 1:2 to give 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F) as white needles (mp 124-125°) (yield = 1% !). On a Merck no. 5714 $60F_{254}$ tlc plate eluted with chloroform DBD-F has Rf 0.32 and lies between two other reaction products (Analyst 1989, 114, 413). It is also reported that DBD-F can be purchased from Tokyo Kasei (TCI America, Portland OR). Add 123 mg 4-(N,N-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole in 20 mL MeCN dropwise to 129 mg piperazine in 20 mL MeCN at room temperature, stir for 30 min, evaporate under reduced pressure, dissolve residue in 50 mL 5% HCl, wash three times with 20 mL ethyl acetate, discard ethyl acetate extracts, adjust pH of aqueous solution to 13-14 with 5% NaOH, extract five times with 50 mL ethyl acetate, combine extracts, wash with 20 mL water, dry over anhydrous sodium sulfate, evaporate under vacuum to give 4-(N,N-dimethylaminosulfonyl)-7-N-piperazino-2,1,3-benzoxadiazole as orange crystals (mp 121-2°).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Inertsil ODS-2
Mobile phase: MeCN:water 50:50
Column temperature: 40
Flow rate: 1
Injection volume: 1
Detector: F ex 437 em 561

CHROMATOGRAM
Retention time: 17
Limit of detection: 13 fmol

OTHER SUBSTANCES
Simultaneous: dehydrocholic acid

KEY WORDS
derivatization

REFERENCE
Toyo'oka,T.; Ishibashi,M.; Takeda,Y.; Nakashima,K.; Akiyama,S.; Uzu,S.; Imai,K. Precolumn fluorescence tagging reagent for carboxylic acids in high-performance liquid chromatography: 4-substituted-7-aminoalkylamino-2,1,3-benzoxadiazoles, *J.Chromatogr.*, **1991**, *588*, 61–71.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in MeCN:0.8 M NaOH 8:92, inject a 25 μL aliquot.

HPLC VARIABLES
Guard column: CarboPac PA-100 (Dionex)
Column: 250 × 4 8.5 μm CarboPac PA-100 (Dionex)
Mobile phase: MeCN:water 15:85 containing 900 mM sodium acetate and 100 mM NaOH
Flow rate: 0.8
Injection volume: 25
Detector: E, Dionex PAD-2 pulsed amperometric detector, gold working electrode, V1 + 0.05 V, t1 480 ms, V2 + 0.60 V, t2 120 ms, V3 -0.60 V, t3 60 ms

CHROMATOGRAM
Retention time: 5.87

OTHER SUBSTANCES
Simultaneous: chenodiol, deoxycholic acid, cholic acid, glycocholic acid, taurocholic acid, glycodeoxycholic acid, glycodeoxychenodeoxycholic acid, ursodeoxycholic acid, taurodeoxycholic acid, taurochenodeoxycholic acid, glycolithocholic acid, lithocholic acid, taurolithocholic acid

REFERENCE
Chaplin,M.F. Analysis of bile acids and their conjugates using high-pH anion-exchange chromatography with pulsed amperometric detection, *J.Chromatogr.B*, **1995**, *664*, 431–434.

SAMPLE
Matrix: solutions
Sample preparation: Mix 200 μL of a solution of bile acids with 50 μL 2.1 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, add 300 μL 10 mM tetrakis(decyl)ammonium bromide in 100 mM pH 7.0 phosphate buffer, heat at 40° for with sonication 10 min, add 300 μL 5.1 μM IS in MeCN, sonicate at room temperature for 1 min, inject a 50 μL aliquot. (Prepare 2-bromoacetyl-6-methoxynaphthalene by stirring equimolar amounts of 2-acetyl-6-methoxynaphthalene (Janssen Chimica, Belgium) and phenyltrimethylammonium tribromide in THF at room temperature for 3 h (Phosphorus and Sulfur 1985, 25, 357), purify by column chromatography on silica gel with chloroform:petroleum ether 50:50 (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES
Column: 250 × 4.6 Ultracarb 5 ODS
Mobile phase: Gradient. A was water. B was MeCN:MeOH 60:40. A:B 55:45 for 20 min, to 30:70 over 10 min, maintain at 30:70 for 25 min, return to initial conditions over 5 min.
Column temperature: 35
Flow rate: 1.2
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM
Retention time: 13
Internal standard: 6-methoxynaphthacyl ester of lauric acid (36)
Limit of detection: 1-2 pmole

OTHER SUBSTANCES
Simultaneous: chenodiol, cholic acid, deoxycholic acid, lithocholic acid

KEY WORDS
derivatization

REFERENCE
Gatti,R.; Roda,A.; Cerre,C.; Bonazzi,D.; Cavrini,V. HPLC-fluorescence determination of individual free and conjugated bile acids in human serum, *Biomed.Chromatogr.*, **1997**, *11*, 11–15.

SAMPLE
Matrix: urine
Sample preparation: Centrifuge urine, pass 40 mL urine through a pre-washed C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate to dryness and take up the residue in 10 mL 100 mM pH 5.0 sodium acetate buffer, add 100 μg β-glucuronidase, add 100 μg cholylglycine hydrolase, heat at 37° for 36 h, pass the mixture through a pre-washed C18 Sep-Pak SPE cartridge, wash with 10 mL water, elute with 10 mL MeOH. Evaporate to dryness and take up the residue in 1 mL MeOH, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 37-50 μm Corasil C18
Column: 100 × 85 μm μBondapak C18 radial pack
Mobile phase: MeCN:MeOH:water:acetic acid 70:20:70:1
Flow rate: 2
Injection volume: 50
Detector: RI

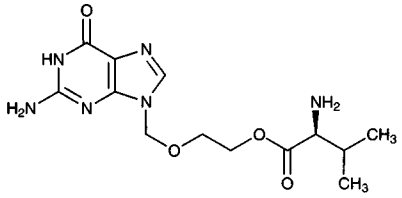
CHROMATOGRAM
Retention time: 11
Limit of detection: 1000 ng

OTHER SUBSTANCES
Extracted: chenodiol, bile acids, deoxycholic acid

KEY WORDS
SPE

REFERENCE
Batta,A.K.; Shefer,S.; Batta,M.; Salen,G. Effect of chenodeoxycholic acid on biliary and urinary bile acids and bile alcohols in cerebrotendinous xanthomatosis; monitoring by high performance liquid chromatography, *J.Lipid Res.*, **1985**, *26*, 690–698.

Valacyclovir



Molecular formula: $C_{13}H_{20}N_6O_4$
Molecular weight: 324.34
CAS Registry No.: 124832-26-4, 124832-27-5 (HCl)
Merck Index: 10039

SAMPLE
Matrix: tissue
Sample preparation: Blend 20% intestinal tissue in Ringers buffer at high speed for 2 min. Centrifuge the homogenate at 12000 g for 15 min at 4°. Remove a 50 μL aliquot of the supernatant, add 450 μL stopping solution. Centrifuge at 12000 g for 5 min at 4°, inject an aliquot of the supernatant. (Stopping solution was an ice cold mixture of 200 μL MeOH and 200 μL pH 6.5 buffer. Buffer was 15 mM 2-[N-morpholino]-ethanesulfonic acid (MES), 130 mM NaCl, 5 mM KCl, and 0.01% PEG-3350.)

HPLC VARIABLES
Guard column: 20 mm long Supelguard LC-18S
Column: 250 × 4.6 Supelcosil LC-18S
Mobile phase: Water:buffer 20:80 (Buffer was MeOH:100 mM potassium phosphate monobasic 25:75, adjusted to pH 6.7 with 1 M NaOH. Water:buffer ratio may be adjusted to ensure separation of other compounds.)
Flow rate: 1
Detector: UV 252

CHROMATOGRAM
Limit of quantitation: 1 μM

OTHER SUBSTANCES
Simultaneous: p-aminohippuric acid sodium salt, amoxicillin, ampicillin, cefadroxil, cephradine, formycin B, quinine, stavudine, thymidine, valine

KEY WORDS
rat; intestine

REFERENCE
Sinko,P.J.; Balimane,P.V. Carrier-mediated intestinal absorption of valacyclovir, the L-valyl ester prodrug of acyclovir: 1. Interactions with peptides, organic anions and organic cations in rats, *Biopharm.Drug Dispos.*, **1998**, *19*, 209–217.